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A simple apparatus for arrayed preparation of YAC DNA for pulsed-field gel analysis

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▼Yeast artificial chromosomes (YACs) have been widely used in the mapping of the human genome and the isolation of human disease genes. YAC contigs are now available to cover nearly the entire human genome (Ref. 1), but the depth of these contigs is often not sufficient to allow high-resolution ordering of markers. YAC fragmentation (Ref. 2, 3, 4), which generates a series of deletions from one end, is a simple and quick method for refined mapping of existing YAC contigs. YAC fragmentation also provides the possibility of discarding the non interesting parts of a YAC, which is useful for subsequent studies such as restriction mapping, subcloning, gene searching, gene characterization and YAC-derived transgenes. However, the task of analysing a large number of fragmented YAC products is both laborious and tedious. Fortunately, a method that enables the high-throughput preparation of YAC DNA for pulsed-field gel analysis has been described by Markie (Ref. 5). This simple and reliable methods allows a single operator to process several plates of 96 YACs in parallel. The main obstacle that prevents the wide use of this method, however, is that the apparatus is not available commercially. Preparing Markie's 96-well template (formed by filing square the round holes of a tip box plastic insert) is very labour-intensive. The resulting rough surface of the template also tends to retain the formed agarose plugs and consequently their removal from the filter surface causes errors in the assignment of sites to plug co-ordinates. Here, we describe the use of a plastic 96-well plate from the MultiScreen Assay System (Millipore; MAGV N22 10) to solve this problem. The MultiScreen Assay System consists of a 96-well plate, a lid and a soft plastic base. We make use of this 96-well plate as a mould for the arrayed YAC DNA preparation. The membranes at the bottom of the 96-well plate are removed simply by placing sticky tape on top of it and tearing the tape away together with the membranes. The plate is placed upside down on a transparent acrylic plate (13 × 9 × 1 mm) and clamped in place by four bulldog clips (Fig. 1). The other advantage of using this apparatus is that the formed agarose plugs are very easily detached from the smooth surface of the plastic 96-well template.

The YAC DNA is prepared the same way as described by Markie (Ref. 5). In short, the YAC clones stored in a standard 96-well storage plate are thawed, the yeast suspension is transferred with a 96-pin (3 mm pin diameter) stainless steel device (Denley) from the wells to the surface of a 7.8 × 11.9 cm Hybond-N filter (Amersham; RPN. 118N) which is placed on top of an agar plate. The growth plate is incubated at 30°C for 2 days. The filter is then lifted from the agar and placed in a rectangular Petri dish [colony picker plate (Hybaid)] on a sheet of 3MM paper (Whatman) saturated with spheroplasting solution [1 M sorbitol, 20 mM EDTA, 10 mM Tris–HCl, pH 7.40, containing 20 units/ml yeast lytic enzyme (ICN; 3 60951) and 30 mM β-mercaptoethanol]. The filter is incubated at 37°C for 30 min and then lifted from the dish and placed on two layers of Parafilm on the surface the acrylic plate. The 96-well plate with its membranes removed is clamped in place over the yeast patches on the filter. Spheroplasting solution is dropped into each of the 96 wells that are formed (20 μl/well). Then 60 μl of 2% molten LMP agarose (made up in spheroplasting solution) is added to eight wells at a time using a multi-channel pipettor, and the yeast cells

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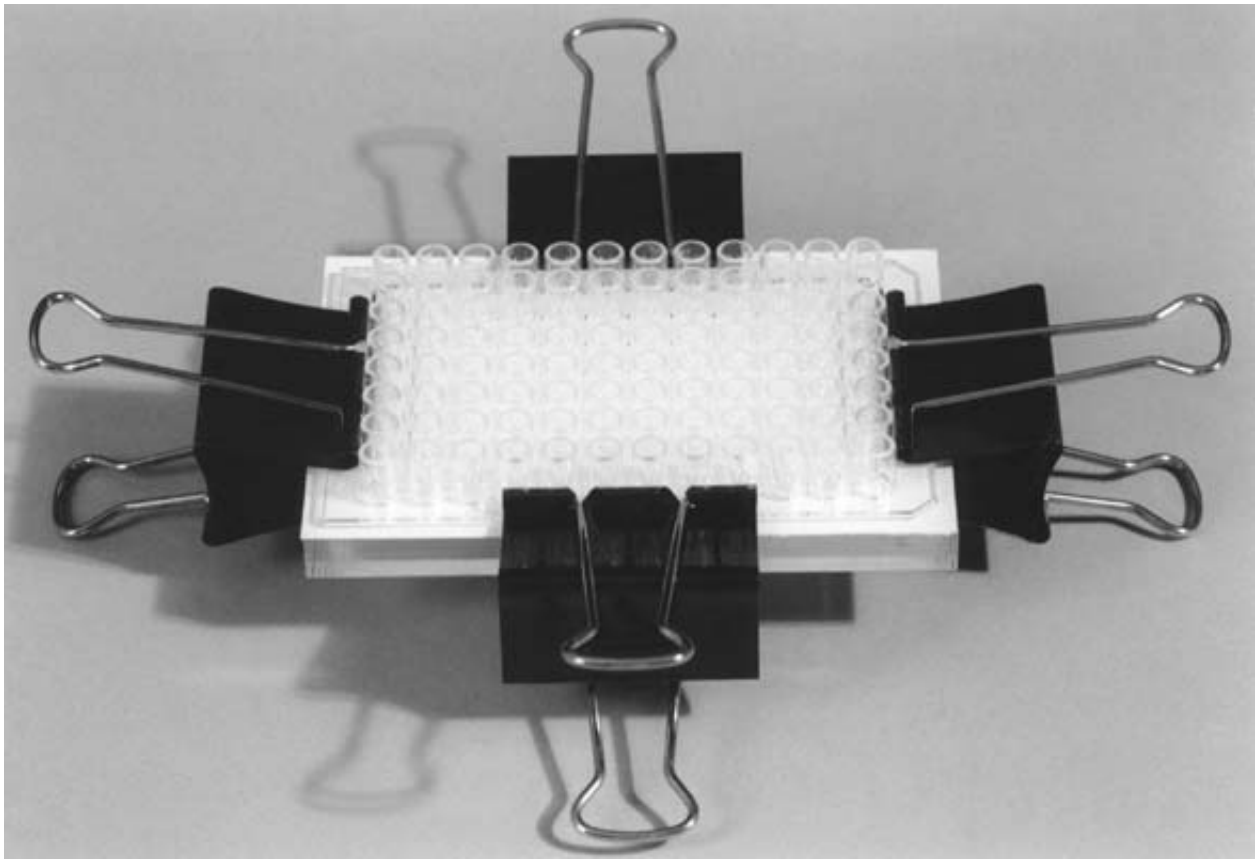

FIGURE 1. The block-forming apparatus. The plastic 96-well plate is part of the MultiScreen Assay System (Millipore). The membranes at the bottom of the 96-well plate are removed before use. The plate is placed upside down on top of the filter carrying the arrayed yeast colonies and clamped to the transparent acrylic plate (13 × 9 × 1 mm) with four bulldog clips.

are evenly suspended by stirring briskly with the tips. The agarose is allowed to set (it is better to leave the agarose to set at 4°C for 20 min as then the agarose blocks detach easily from the 96-well template) and then the template is gently eased from the filter, leaving the agarose blocks attached to the filter in an 8 × 12 array. The filter is then placed in an empty rectangular Petri dish and sufficient spheroplasting solution is added to submerge the blocks (to prevent the agarose plugs from detached from the filter, it is advisable not to move the preparation around when they are submerged in the buffer). After 3–4 h incubation at 37°C, the solution is then carefully changed for a similar volume of yeast lysis solution [1% (w/v) lithium dodecyl sulfate, 100 mM EDTA, 10 mM Tris–HCl pH 8.0] and incubated for a further 30 min at 37°C. The yeast lysis solution is then changed for fresh solution and the plate incubated overnight at 37°C. Plates, sealed with tape, are stored at room temperature with the blocks immersed in yeast lysis solution, until required.

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Products Used

Hybond N: Hybond N from Amersham Pharmacia Biotech

colony picker plate: colony picker plate from Hybaid Limited

3MM paper: 3MM paper from Whatman Inc